REMARKS

Claims 1-44 are cancelled. Claim 52 remains in the application. Claims 45-51 and 53-54 are withdrawn from consideration by the Examiner. No new matter has been entered.

I. ELECTION/RESTRICTIONS

The Examiner acknowledges agreeing to examine an additional sequence. Applicant added the novel sequence SEQ ID No.: 329 to the combination comprising SEQ ID No.: 115-124 that the Examiner had previously examined. Claims 47, 48, 49, 50, 51, 52 and 53 includes Applicant's original election of Group IV sequences (SEQ ID No.: 115-124). Claims 48, 51, 52, and 53 include the newly added sequence in addition to the Group IV sequences that the Examiner has already examined. In a telephone interview on June 22, 2004, the Applicant elected claim 52 with traverse in light of the Examiner's request to make an election of a single combination. The Examiner has withdrawn claims 45-51 and 53-54 as being directed to a non-elected invention.

As discussed during the phone interview on June 22, 2004, the Restriction should not be maintained as to claims 48, 51, 53 and 54 for the following reasons. First, the Examiner need not examine the entire combination since the Applicant identified the sequence that Applicant believes to be novel (SEQ ID No.: 329). MPEP §803.04 states that if a single novel sequence in a combination of sequences is found to be novel then the entire

combination is novel. Since SEQ ID No.: 329 is in each of claims 47, 48, 50, 51, 52 and 53 there is no additional work for the Examiner to examine these claims since the sequence combination is novel via the addition of SEQ ID. No. 329. Further still, claim 53 depends from independent claim 52. Claim 54 incorporates claim 53 in its entirety and is connected in design operation and effect. Examination of claims 53 and 54 will not cause additional search or work for the Examiner and therefore should not be restricted from examination in the present application.

II. UTILITY UNDER 35 U.S.C. §101

The Examiner has rejected claim 52 under 35 U.S.C. §101 because the Examiner states that "the claimed invention is not supported by either specific asserted utility or a well established utility". Applicant traverses the Examiner's utility rejection of claim 52 for the following reasons. First, the Examiner's position on this point is at odds with prior patents issued by the USPTO for toxicity screening. Patent 6,160,105 titled "Monitoring Toxicological Responses" was filed 10/13/1998 and issued 12/12/2000 for the sole utility of detecting a toxicological effect of a test compound associated with increased or decreased levels of a polynucleotide sequence in a sample. Patent 6,228,589 titled "Measurement of Gene Expression Profiles in Toxicity Determination" was filed 4/7/1997 and issued 5/8/2001

for the sole utility of assessing the toxicity of a compound in a test organism by measuring gene expression profiles of selected tissues. Patent 6,372,431 titled "Mammalian Toxicological Response Markers" was filed 11/19/1999 and issued 4/16/2002 for the sole utility of detecting metabolic and toxicological responses with array elements in a microarray. Patent 6,403,778 titled "Toxicological Response Markers" was filed 8/28/1998 and issued 6/11/2002 for the sole utility of screening compounds and therapeutic treatments for toxicological responses using a composition used as hybridizable array elements in a microarray. A utility of Applicant's invention is directed to detecting toxicological responses with array elements in a microarray and detecting toxicological effect of a test compound associated with increased or decreased levels of a polynucleotide sequence in a sample. Therefore, Applicant's invention demonstrates a specific asserted utility and a well established utility as is also demonstrated for the U.S. patents 6,160,105; 6,228,589; 6,372,431; and 6,403,778.

Second, even if the Examiner does not recognize the PTO's acknowledged well established and credible utility of monitoring toxicity responses, Applicant's invention is useful for monitoring the health of a canine. For example, SEQ ID No.:116 represents a partial gene sequence of the c-erbB2 gene. Overexpression of c-erbB-2 is known to correlate with more rapid

progression and a worse prognosis in breast cancer and other tumors of canines. See "The canine ERBB2 gene maps to a chromosome region frequently affected by aberrations in tumors of the dog" Cytogenetic and Genome Research, 2001;**94**:194-195, abstract. SEQ ID No.:117 represents a partial gene sequence for the catalase gene. Catalase is known to change expression in lung cancer. See "Differential Expression of Manganese Superoxide Dismutase and Catalase in Lung Cancer" Cancer Research 2001;**61**:8578-8585, introduction. SEQ ID No.:118 represents a partial gene sequence of p53 gene. p53 is known to be overexpressed in canine osteosarcomas and has been correlated with highly aggressive tumor behavior. See "p53 tumor suppressor protein overexpression in osteogenic tumors of dogs", Vet. Path. 1996;**33**:213-221, abstract. SEQ ID No.:121 represents a partial gene sequence for metallothionein-1. Metallothionein-1 is known to change expression in lung cancer where overexpression of Metallothionein-1 is indicative of short term survivability. See "Metallothionein expression in patients with small cell carcinoma of the lung, correlation with other molecular markers and clinical outcome" Cancer 2001;**92**:836-842. SEQ ID No.:123 represents a partial gene sequence of Multidrug Resistant Protein-1. Overexpression of Multidrug Resistant Protein-1 is predictive of tumor progression and is a prognostic indicator of

outcome for many cancer/tumors. See "Expression of the Gene for Multidrug-Resistance-Associated Protein and Outcome in Patients with Neuroblastoma" NEJM 1996;**334**:231-238. SEQ ID No.:329 is a partial gene sequence of a gene that changes expression in response to toxic doses of etoposide, aspirin and caffeine (Table 9). It is well known in the art that both etoposide and aspirin are commonly used to treat canines for cancer and pain management. Caffeine is a common ingredient in chocolate which is known to be toxic to canines. Therefore, the combination of SEQ ID. No.: 329 and 116, 117, 118, 121, and 123 are useful for monitoring the health of a canine.

Third, Applicant agrees with the Examiner's position "that many genes are irrelevant in gene microarray assays" which is supported by Li *et al*. This is precisely the reason that Applicant screened a substantial portion of coding genes to identify through differential gene expression, in silico consulting and identification of toxicologically relevant canine sequences (not in public databases such as GeneBank) those genes which were responsive to toxic agents in a canine or canine cell culture. The discovery of toxicologically relevant genes is clearly stated throughout the application such as in the Background of the Invention section at paragraph 9, the Summary of the Invention section at paragraph 11 and in methods of identifying toxicologically relevant genes at paragraphs 73-81.

For example, open-ended approaches to discover and identify toxicological responsive genes are described in paragraph 74, and paragraphs 77-79. Specifically, these approaches selectively identify genes whose expression is modulated by toxic processes. In these approaches canine cells or canines are exposed to treatments with compounds at doses documented to produce toxicity. Expression of a large number of genes (an appreciable fraction of the entire genomic expression profile) is evaluated by open-ended expression profiling technology such as differential display. Those genes which have appreciable differences in expression in treated compared to untreated (control) samples are identified as toxicologically relevant. Although the technologies visualize expressions of thousands of genes only a small subset are identified as responsive to toxic treatments. Subsequent cloning and sequencing of these toxicologically responsive genes permits identification of the sequences of genes which have not previously been identified as being toxicologically responsive and sequences which are not described in the public domain and are toxicologically responsive.

An example of these open-ended discovery processes are provided in Example 4 at paragraph 120 for cultured canine cells. The application clearly teaches the advantage of identifying and utilizing subsets of informative gene sequences rather than

utilizing large collections of sequences of which many are not informative.

Additionally, gene sequences were identified using primers derived from publicly available sequences of toxicologically relevant genes from species other than canine, and the canine toxicologically relevant sequences where selected for arrays based upon the canine sequences toxicological responsiveness.

Finally, Applicant has deleted the term "toxicologically relevant" in claim 52. The amendment places claim 52 in condition for allowance.

III. Rejection of claim 52 under 35 U.S.C. § 112 paragraph 1

The Examiner states that claim 52 is rejected for failing to comply with the enablement requirement because the claim contains subject matter which was not descried in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and or use the invention. The Examiner questions whether the change in differential gene expression of treated versus untreated samples for genes of interest is significant.

Applicant traverses the Examiner's rejection for the following reasons. First, there are abundant examples of statistical analysis approaches known in the art to evaluate differential expression. Several examples of microarray data from arrays prepared with canine sequences are included in the

application. Specific examples of such microarray data are included by reference in Table 11 and figures 1-5. These data produced from "two-color" microarrays which have been probed with two different fluorescent probes prepared from treated and untreated cells, tissues or organs (See Detailed Description of the Invention section at paragraph 104, Examples 1-3, Examples 9-12).

For example, fluorescent data from arrays hybridized with treated and control probes can be measured with any of a number of array scanners, including scanners such as an Axon array scanner which was used in the examples of this application. The arrays contain replicate spots for each gene sequence with fluorescent signals from probes prepared from treated and control samples. Data from these replicate spots are analyzed by several standard statistical analysis procedures to determine whether statistically significant differential expression has been observed for each gene sequence. Hundreds of published references are available describing robust procedures for differential expression statistical analysis. Examples of specific procedures include analysis of variance (Arfin, S. M., A. D. Long, et al., "Global gene expression profiling in Escherichia coli K12. The effects of integration host factor." J Biol Chem 2001;**275**:29672-84.), t-tests using observed variance or Bayesian estimates of variance (Long, A. D., H. J. Mangalam, et

al., "Improved statistical inference from DNA microarray data using analysis of variance and a Bayesian statistical framework. Analysis of global gene expression in Escherichia coli K12." J Biol Chem 2001;**276**:19937-44.), Students t-test with beta-binomial distribution within slides and gamma-Poisson between slides (Baggerly, K. A., K. R. Coombes, et al., "Identifying differentially expressed genes in cDNA microarray experiments." J Comput Biol 2001;**8**:639-59) and Bayesian procedures (Lee, M. L., W. Lu, et al. "Models for microarray gene expression data." J Biopharm Stat 2002;**12**:1-19). General references presenting and comparing analysis technologies include Lee, M. L., W. Lu, et al. "Models for microarray gene expression data." J Biopharm Stat 2002;**12**:1-19; Nadon, R. and J. Shoemaker "Statistical issues with microarrays: processing and analysis." Trends Genet 2002;**18**:265-71.

In addition to published statistical analysis procedures there are a number of commercial software packages available which provide capabilities for statistical analysis of gene expression. These commercial software products include capabilities of analyzing "two-color" array data produced with an Axon scanner and GenePix software (as used for Table 11 and Figures 1-5). Examples of such software are the Insight S+ArrayAnalyzer™ (Insightful, Seattle WA) which provides

capabilities for statistical analysis of differential expression for GenePix files using several methods (two sample and paired t-test, one way ANOVA and Wilcoxon) and GeneSpring (Agilent Technologies/Silicon Genetics, Redwood City, CA) which has several differential expression statistical analyses (t-tests, 2 way ANOVA and one way post hoc tests) that can be applied to a variety of microarray formats including Axon/GenePix produced files.

The Applicant used the GenePix software and at least one of the above described methods well known in the art at the time the application was filed in determining statistically significant changes in expression for the genes under review.

In addition to differential expression evaluation, Applicant utilized comparison evaluation in comparing expression profiles, from exposure to a test sample to profiles in a database of expression profiles from samples treated with toxicants. This analysis is referenced in the patent application in several places (Summary of the Invention, section at paragraph 14, 16 and 17; Detailed Description of the Invention section at paragraph 107 and 108). The utility of this concept is clearly supported for toxicogenomics application of arrays and array analysis. (Farr and Dunn, "Concise Review: Gene Expression applied to Toxicology" Toxicological Sciences 1999;**50**:1-9, page 4 Interpretation of Gene Expression Data).

In the wake of the publication of "Gene Expression applied to Toxicology", others have identified toxicologically relevant genes. More specific examples of the use of correlative analysis of microarray data for toxicology purposes illustrate the availability of knowledge and teaching in this area. Waring *et al.* compared microarray-derived gene expression profiles from livers of rats treated with hepatoxicants. See "Clustering of hepatotoxins based on mechanism of toxicity using gene expression profiles" Toxicol Appl Pharmacol 2001;**175**:28-42). Their analyses employed a variety of clustering algorithms including hierarchical, divisive hierarchical, k-means partitioning and self-organizing maps. This analysis permitted identification of clustered expression profiles which correlated with histopathological changes, serum chemistry changes and mechanisms of hepatotoxicity. This work clearly illustrated the potential for using gene expression profiles and a database to obtain predictive capability for a variety of toxicologically significant properties.

Another specific example of the utility of comparative analysis of microarray data for toxicology purposes is provided in Burczynski, McMillian et al. "Toxicogenomics-Based Discrimination of Toxic Mechanism in HepG2 Human Hepatoma Cells", Toxicological Sciences 2000;**58**:399-415. In this study, cultured

human hepatic cells were exposed to a variety of toxicants with different mechanisms and a database of microarray expression profiles was obtained. The main correlation statistic used for analysis of the data was the Pearson correlation coefficient. The analysis enabled identification of subsets of genes on the microarray whose expression distinguished between DNA-damaging and inflammatory agents. Comparison of single microarray data for agents not included in the training set demonstrated the capability to classify many of these agents as correlating with either a DNA-damaging or inflammatory pattern of expression. This study clearly teaches and demonstrates the potential for developing microarray expression databases and comparative analysis procedures for toxicology applications.

Further, it is well known in the art that comparison of gene expression profiles can be accomplished by correlative statistical analysis for all or subsets of multiple gene expressions on a microarray without evaluation of the differential expression significance of each individual gene. Overviews of statistical analysis procedures for this purpose are provided in numerous references such as Hoffmann, R., T. Seidl, et al. "Profound effect of normalization on detection of differentially expressed genes in oligonucleotide microarray data analysis" Genome Biol 2002;**3**(7):RESEARCH0033.

Common statistical procedures have been applied to

comparative analysis of microarrays and include: correlation coefficient statistics such as the Pearson correlation. (Wall, M. E., P. A. Dyck, et al. "SVDMAN--singular value decomposition analysis of microarray data." Bioinformatics 2001;**17**(6):566-8), Spearman correlation (Kotlyar, M., S. Fuhrman, et al. "Spearman correlation identifies statistically significant gene expression clusters in spinal cord development and injury." Neurochem Res 2002;27(10):1133-40) a variety of cluster analysis approaches including self-organizing map statistics (Chen, J. J., K. Peck, et al. "Global analysis of gene expression in invasion by a lung cancer model." Cancer Res 2001;61:5223-30.), graphical Gaussian modeling (Toh, H. and K. Horimoto "Inference of a genetic network by a combined approach of cluster analysis and graphical Gaussian modeling." Bioinformatics 2002;18:287-97), and heuristic two-step clustering (De Smet, F., J. Mathys, et al. "Adaptive quality-based clustering of gene expression profiles." Bioinformatics 2002;18:735-46.), hierarchical and principal component analysis (Peterson, L. E. (2002). "CLUSFAVOR 5.0: hierarchical cluster and principal-component analysis of microarray-based transcriptional profiles." Genome Biol 2002;**3**:SOFTWARE0002).

Applicant used at least one of the correlative statistical analysis methods well known in the art at the time the application was filed to comparatively analyze the test sample

against a reference sample.

Therefore all of the claims presented are enabled and in condition for allowance.

IV. Rejection of claim 52 under 35 U.S.C. §112 paragraph 2

The Examiner states that claims 52 is rejected under 35 U.S.C. 112, paragraph 1, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s) at the time the application was filed, had possession of the claimed invention. Applicant has amended claim 52 to add the term "consisting of" SEQ ID No.: 329 to the combination of SEQ ID No.: 116, 117, 118, 119, 121, and 123. Therefore the amendment overcomes the Examiner's rejection and places claim 52 in condition for allowance.

V. CLOSING

In view of the above, Applicant respectfully submits that independent claim 52 is patentable over the prior art. Applicant further submits that dependent claim 53 is patentable, at least as being dependent from patentable independent claim 52 and are further patentable due to the additional limitations recited therein.

Applicant has included an Appendix with the references cited in section II and III of this amendment for the Examiner's reference.

For the above reasons, Applicant respectfully submits that the application is in condition for allowance with claims 52-53. If there remain any issues that may be disposed of via a telephonic interview, the Examiner is kindly invited to contact the undersigned at the exchange given below.

The Director of the Patent and Trademark Office is authorized to charge any necessary fees, and conversely, deposit any credit balance, to Deposit Account No. 13-4213.

Respectfully submitted,

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APPENDIX

"The canine ERBB2 gene maps to a chromosome region frequently affected by aberrations in tumors of the dog" Cytogenetic and Genome Research, 2001;**94**:194-195, abstract

"Differential Expression of Manganese Superoxide Dismutase and Catalase in Lung Cancer" Cancer Research 2001;**61**:8578-8585

"p53 tumor suppressor protein overexpression in osteogenic tumors of dogs", Vet. Path. 1996;**33**:213-221, abstract

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